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# Synthesis and Biological Evaluation of Cannabinoid Receptor Ligands

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SYNTHESIS AND BIOLOGICAL EVALUATION OF CANNABINOID RECEPTOR  
LIGANDS

by  
Mariah Leigh Cole

A thesis submitted to the faculty of The University of Mississippi in partial  
fulfillment of the requirements of the Sally McDonnell Barksdale Honors  
College.

Oxford, MS  
April 12, 2017

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I would like to acknowledge the Sally McDonnell Barksdale Honors College and The University of Mississippi School of Pharmacy for all of the opportunities they have offered me to further my education. The research described in this thesis was supported by the National Institute of General Medical Sciences (NIGMS) Grant Number P20GM104932: COBRE, CORE-Natural Products Neuroscience, Research Core B, and would not have been possible without receiving a 2015 NIH Fellowship through the University of Mississippi COBRE Summer Undergraduate Research Program.

Finally, I owe my deepest gratitude to my family and friends for their constant love and support.

## ABSTRACT

The cannabinoid receptors, members of the G-protein coupled receptor (GPCR) superfamily, have been implicated in numerous human physiological functions and diseases. These receptors, cannabinoid type 1 (CB<sub>1</sub>) and cannabinoid type 2 (CB<sub>2</sub>), are most concentrated in the central nervous system and immune cells, respectively, and have each become a target of therapeutic interest. Dual CB<sub>1</sub>/CB<sub>2</sub> agonists such as delta-9-tetrahydrocannabinol (THC) have demonstrated efficacy in the treatment of nausea, pain, and glaucoma, but suffer from psychotropic effects mediated by CB<sub>1</sub>, motivating the search for CB<sub>2</sub> selective therapeutic agents. Selective modulation of the CB<sub>2</sub> receptor has therapeutic potential in human health disorders such as pain, inflammation, and cancer. In our efforts to develop CB<sub>2</sub> receptor selective ligands, we preliminarily examined a structure activity relationship (SAR) study of synthetic and natural terpenoid cannabinoids to design more potent and selective CB receptor ligands. In an effort to expand this SAR, we synthesized a series of analogs with alternative functional groups and substitution patterns using a dihydrobenzofuran scaffold, with previous biological assay data guiding the design of our new compounds. Aldol condensation and Luche reduction reactions were used to create six new analogs. The structures of the new analogs synthesized were confirmed using NMR and MS techniques. The compounds were submitted for biological evaluation

in a radioligand displacement assay for both the CB<sub>1</sub> and CB<sub>2</sub> receptors. One compound exhibited modest affinity for the CB<sub>2</sub> receptor. The alterations in functional groups and substitution patterns provided analog data to help create a more comprehensive structure activity relationship study in the future development of CB<sub>2</sub> selective compounds. *This research was funded by Grant Number P20GM104931 from the National Institute of General Medical Sciences (NIGMS), COBRE-NPN.*

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## LIST OF ABBREVIATIONS AND SYMBOLS

2-AG	2-arachidonoylglycerol
10a-OH THC	(-)-alpha-10a-hydroxy-delta-9-tetrahydrocannabinol
ACN	acetonitrile
AEA	N-arachidonoyl ethanolamide
Ar	argon
BuLi	butyllithium
CB	cannabinoid
CB <sub>1</sub>	cannabinoid receptor subtype 1
CB <sub>2</sub>	cannabinoid receptor subtype 2
CBD	cannabidiol
CBN	cannabinol
CNS	central nervous system
DMF	<i>N, N</i> -dimethylformamide
DMSO	dimethylsulfoxide
EDDA	ethylenediamine acetate
ESI	electrospray ionization
GPCR	G-protein coupled receptors
NMR	Nuclear Magnetic Resonance
Pd/C	Palladium on carbon

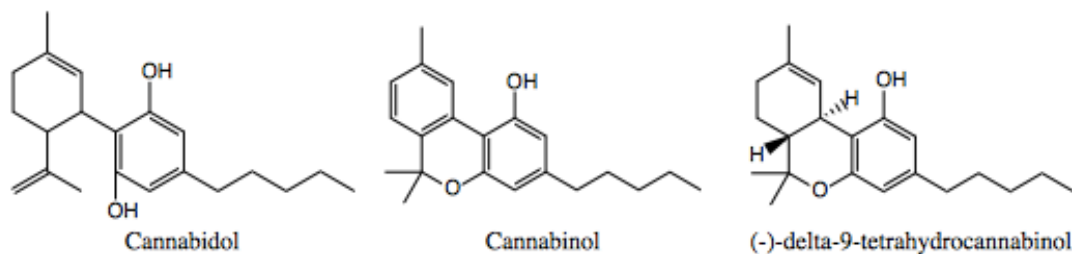
SAR	structure activity relationship
THC	delta-9-tetrahydrocannabinol
THF	tetrahydrofuran
TLC	thin-layer chromatography
UV	ultraviolet

## INTRODUCTION

### History and Composition of *Cannabis Sativa*

*Cannabis sativa*, a hemp plant originating in Asia, is one of the oldest known mind-altering drug-containing species.<sup>1</sup> Historically, the hemp plant has various uses including weaving its fibers into fabric, producing bowstrings for archers, and manufacturing of paper.<sup>2</sup> During the Sufis movement, cannabis use was reported as a means of communication with Allah; the founders of this Islamic movement believed this was only possible in an ecstatic state. Thus, as the popularity of the Sufis movement increased so did the drug usage. Now some 200-300 million people are estimated to use cannabis worldwide<sup>1</sup> and in the United States usage may increase due to its legalization and availability as a social drug. With such widespread use of cannabis, researchers were inclined to study its affects and components to determine its safety and therapeutic potential. Thus, since 2012, 545 constituents have been identified with 441 defined as non-cannabinoid and 104 as cannabinoids. Eleven types of cannabinoids or phytocannabinoids found in the resin and leaves of *Cannabis sativa* have been classified as follows: (-)-delta-9-*trans*-tetrahydrocannabinol ( $\Delta^9$ -THC), (-)-delta-8-*trans*-tetrahydrocannabinol ( $\Delta^8$ -THC), cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND) cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), cannabitriol (CBT) and miscellaneous-type cannabinoids.<sup>3</sup> The three

main components and more thoroughly researched components of *Cannabis sativa* are CBD, CBN and THC (**Figure I**).<sup>1, 4</sup>

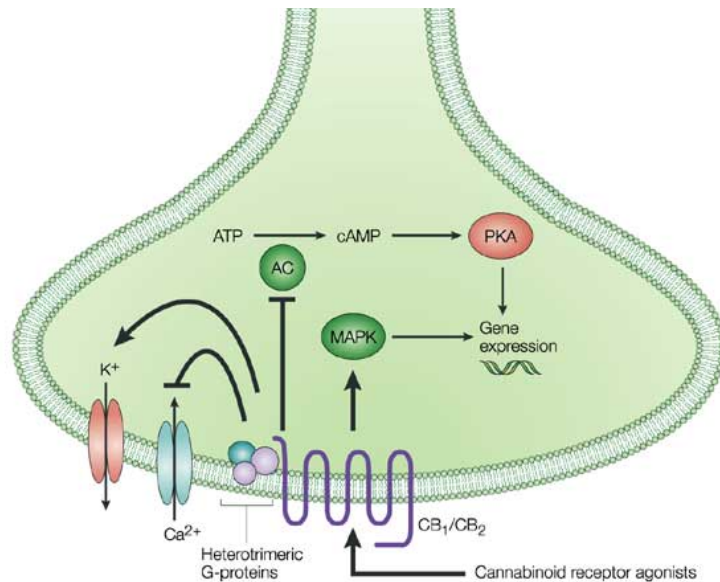


**Figure I. Structures of CBD, CBN, and THC**

### The Endocannabinoid System

The cannabinoid components of cannabis are known to interact with the endocannabinoid system, an important and complex signaling system that has regulatory roles for cells throughout the nervous, immune, and other systems.<sup>5</sup> The endocannabinoid system contains two distinct and critical G-protein coupled receptors (GPCRs) described as cannabinoid receptor subtype 1 (CB<sub>1</sub>) and cannabinoid receptor subtype 2 (CB<sub>2</sub>). GPCRs are part of a large protein receptor family which are responsible for signal transduction in cells. They contain seven  $\alpha$ -helical transmembrane spanning region with an extracellular N-terminal and C-terminal cytosolic domain coupled to a G-protein comprised of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. Generally, ligands bind to the receptor causing the subsequent detachment of the  $\beta$  and  $\gamma$  subunits which activate an effector enzyme. The

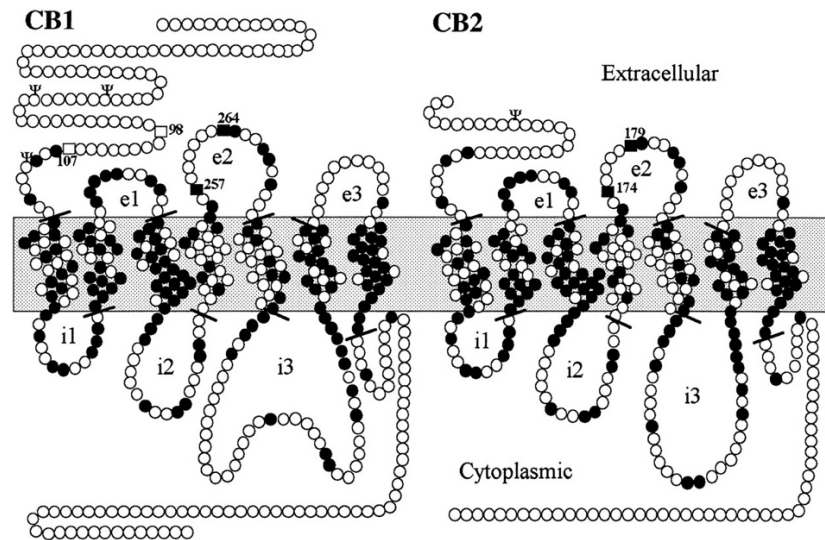
effector enzyme produces second messengers (e.g. cyclic adenosine monophosphate (cAMP), calcium and inositol 1-4-5-triphosphate (IP<sub>3</sub>)).<sup>6</sup> The CB<sub>1</sub> and CB<sub>2</sub> receptors are thought to operate in a more complex manner for signaling which results from the inhibition of adenylate cyclase, specific calcium channels, or activation of protein kinases. This action modulates neurotransmission.



**Figure II. Abbreviated neuronal cannabinoid signaling.** MAPK = mitogen activated protein kinases, AC = adenylyl cyclase, cAMP = cyclic adenosine monophosphate, PKA = protein kinase A.<sup>7</sup>

Both the CB<sub>1</sub> and CB<sub>2</sub> receptors were cloned in 1991 and 1992 respectively and based on their amino acid sequences, were found to be approximately 44% similar (Figure II).<sup>8</sup> The CB<sub>1</sub> receptor is mainly present in central and peripheral neurons, however CB<sub>2</sub> receptors are more profoundly expressed in the spleen and immune cells.

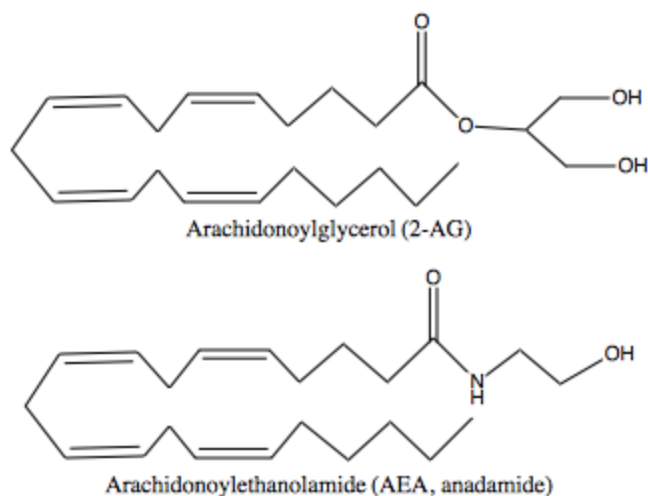
Originally, CB<sub>2</sub> receptors were believed to be relatively absent from the brain, but this was challenged recently. Researchers have found CB<sub>2</sub> protein expression in microglial cells in the brain and CB<sub>2</sub> expression in neurons.<sup>9</sup> However, recent studies<sup>7</sup> cast doubt on this conclusion due to discrepancies in validation of antibodies used in immunolabeling experiments for the CB<sub>2</sub> receptor.



**Figure III. Structure of CB1 and CB2 receptors.**<sup>10</sup>

Discovery of the cannabinoid receptors lead researchers to question whether there were endogenous ligands linked to these receptors. Later research uncovered the presence of endocannabinoids unrelated to the cannabinoid structure found in the hemp plant. These ligands were derivatives of polyunsaturated fatty acids and were identified as anandamide (N-arachidonylethanolamide, AEA), 2-arachidonoylglycerol (2-AG), 2-arachidonoylglyceryl ether (noladin ether), O-arachidonyl-ethanolamine (virodhamine)

and N-arachidonoyl-dopamine (NADA). Anandamide and 2-AG (**Figure III**) were the first ligands discovered and are not produced and stored, but synthesized on demand, then subsequently released.<sup>11</sup> Anandamide is a partial agonist of CB<sub>1</sub> receptor and nearly inactive for the CB<sub>2</sub> receptor. However, 2-AG is a full agonist of both receptors. Interestingly these endocannabinoids also have modest affinity for the vanilloid type-1 (TRPV1) channel.<sup>12</sup> Animal studies have shown that administration of AEA induces hypothermia, analgesia, catalepsy, and appetite stimulation.<sup>9</sup> Although originally considered insignificant initially, 2-AG has been linked to the modulation of feeding, hypotension, neuroprotection and cell proliferation and other interesting physiological processes.<sup>4</sup>



**Figure IV. Structure of endocannabinoids 2-AG and AEA.**

## **Cannabinoid and Endocannabinoid Therapeutic Benefits**

Although the FDA designation of THC and CBD as Schedule I controlled substances may obstruct their development as therapeutics, ample studies have shown these cannabinoids have been shown to offer therapeutic advantages in glaucoma, nausea, AIDS-associated anorexia and wasting syndrome, multiple sclerosis, chronic pain, inflammation and epilepsy.<sup>13</sup> Cannabidiol, marketed as Epidiolex® has not been approved by the US-FDA but is currently under evaluation in a phase III clinical trial for the treatment of seizures in childhood onset epilepsy. In other countries such as Canada and the United Kingdom, Nabiximols (Sativex ®) has been approved as a treatment option for neuropathic pain and disturbed sleep and spasticity in patients with multiple sclerosis. It is administered as an oromucosal spray formulated with THC and CBD extracts and is currently in phase III clinical trials in the US. About 2.5 million people worldwide are affected by multiple sclerosis and without a cure, management of the debilitating symptoms is the goal of treatment.<sup>14</sup> However, problems may arise with providing patients in the US with this treatment option due to federal regulations of THC as a controlled substance. The cannabis extract containing THC is thought to exert its therapeutic effects by activation of either CB<sub>1</sub> or CB<sub>2</sub> receptors.<sup>15</sup> However, the psychoactive effects of cannabis are thought to be due to THC activating the CB<sub>1</sub> receptor. Therefore, studies shifted to targeting the CB<sub>2</sub> receptor to surpass this major hurdle by creating ligands that selectively bind to the CB<sub>2</sub> receptor. Many studies have

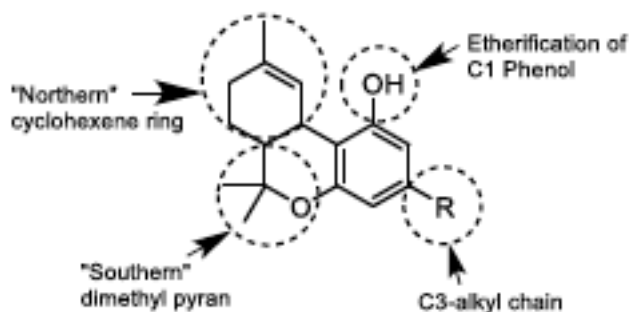


proposed that a CB<sub>2</sub> agonist may be effective in treating a range of conditions and diseases that have a neuro-inflammatory or neurodegenerative component, such as multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, and stroke.<sup>7,16</sup> Therefore, our efforts were directed towards the design and synthesis of an agonist with selectivity for the CB<sub>2</sub> receptor.

## Results and Discussion

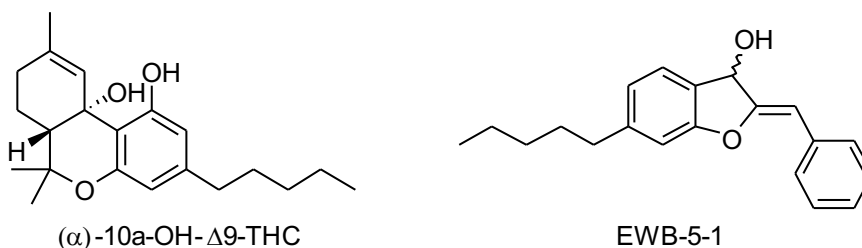
### Design and Synthesis of a Benzofuran Scaffold

My thesis research is a continuation of the graduate dissertation research initially conceived by Eric Bow, Ph.D. at the University of Mississippi. The search for a CB<sub>2</sub> selective modulators began with the design of a scaffold that had spatial similarity to the potent THC analog (-)-alpha-10a-hydroxy-delta-9-tetrahydrocannabinol (10a-OH THC), a natural product isolated and characterized from *Cannabis sativa* by Research Scientists in COBRE-NPN CORE A (unpublished research). This naturally occurring cannabinoid contains a unique hydroxyl substituent at the 10a-position of THC. The analog was found to have high affinity for both the CB<sub>1</sub> and CB<sub>2</sub> receptors. 10a-OH THC was used as a template to create a new scaffold, followed by implementation of a structure activity relationships (SAR) study. In order to design CB<sub>2</sub> selective analogs, several SAR elements derived from the classical cannabinoids (**Figure IV**) were considered, including: i.) etherification of the phenol to decrease CB<sub>1</sub> affinity without effecting CB<sub>2</sub> binding; ii.) variation of ring sizes and substitution patterns in place of the “Northern” cyclohexane ring, and; iii.) substitution in the “Southern” dimethyl pyran ring.

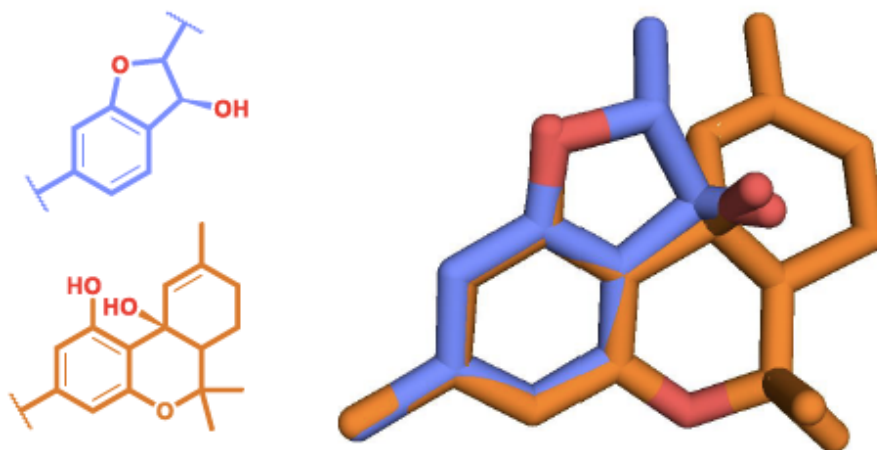


**Figure V. SAR elements of classical cannabinoids.**

These features described in conjunction with molecular modeling tools resulted in the decision to employ a dihydrobenzofuran scaffold, depicted in **Figure VI**.<sup>6</sup> Computational superimposition underlined the similarities in interaction areas of the two molecules (**Figure VII**). The furan oxygen and 3-hydroxy group of the dihydrobenzofuran scaffold overlapped with the phenol and 10a-hydroxy group of 10a-OH THC.<sup>6</sup> This suggested that this novel scaffold may serve as a viable surrogate of the classical cannabinoids and engage in similar binding interactions with CB receptors.

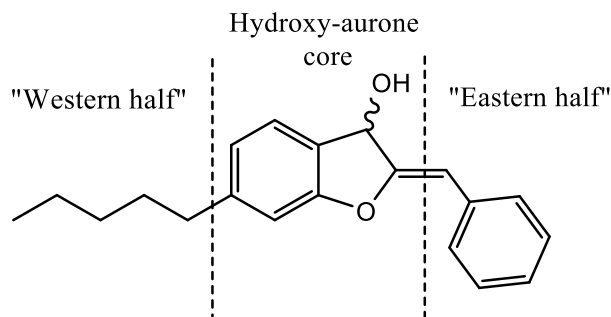


**Figure VI. Structure of (alpha)-10a-OH-delta-9-THC and dihydrobenzofuran analog**



**Figure VII. Computational superimposition of 10a-OH THC and 3-hydroxy-dihydrobenzofuran.** 3D Overlay of 3-hydroxy-dihydrobenzofuran scaffold and 10a-OH THC minimized energy structures in Chem3D 15.0 (Perkin-Elmer). Structures of those used in simulation are shown left in corresponding colors.<sup>6</sup>

Using the dihydrobenzofuran scaffold, a series of analogs were proposed in order to create a small library of compounds for SAR analysis. Each analog was devised by manipulating the “Western half” or “Eastern half” of the 3-hydroxy-dihydrobenzofuran or “hydroxyl-aurone” core (**Figure VIII**). The “Eastern half” was modified by experimenting with the addition of various aryl-containing building blocks to the scaffold by aldol condensation reactions and subsequent Luche reductions. In addition, the “Western half” was altered by changing the nature and position of the alkyl chains. To synthesize these modified ligands, an analog with the desired alkyl chain length, thought to be optimal for CB receptor binding, was synthesized first. This lead compound was then used as a starting point to create several molecules with variations in the phenyl ring of the “Eastern half” using a substituted benzaldehyde in an aldol condensation reaction.



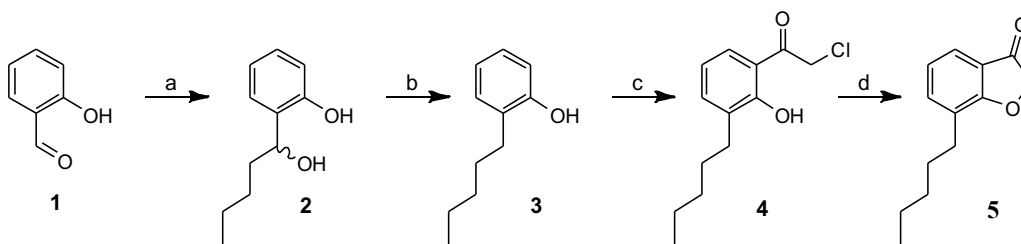
**Figure VIII. Structural map of 3-hydroxy-dihydrobenzofuran scaffold.**

### Synthesis of benzofuran-3(2H)-one

Based on Dr. Bow's synthetic library of compounds synthesized and evaluated for CB binding, it was speculated that more potent and selective CB ligands may be realized with manipulation of alkyl chain substitution, in particularly the regiochemistry of alkyl chain substitution. As a result, we proposed to synthesize analogs containing alkyl chains in the 7 versus the 6 position on the dihydrobenzofuran aromatic ring. This new substituted scaffold would then be subject to subsequent aldol condensation reactions as preformed in previous compound synthesis.

The synthetic protocol for the 7-substituted dihydrobenzofuran scaffold began with the nucleophilic addition of n-butyllithium to commercially available 2-hydroxybenzaldehyde (**1**), which afforded the desired addition product (**2**) in high yield (**Figure IX**). Elimination of the secondary alcohol with catalytic perchloric acid and *in situ* hydrogenation of the resulting styrene gave 2-pentyphenol (**3**). Friedel-Crafts acylation of **3** with chloroacetonitrile under Sugasawa conditions, using  $\text{BCl}_3$  to direct the

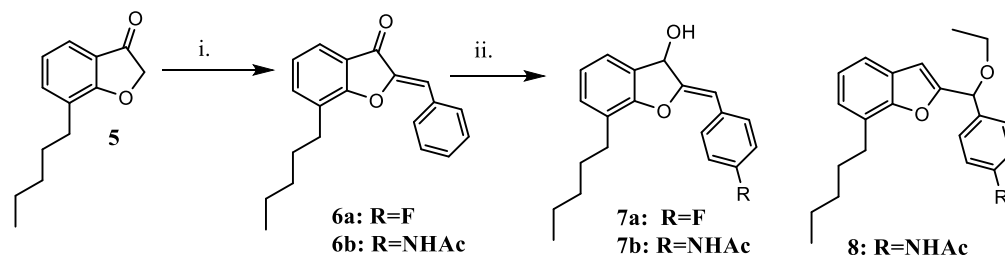
acylation *ortho* to the phenol, afforded the  $\alpha$ -chloro ketone (**4**). The substitution pattern on the aromatic ring of **4** was confirmed by evaluation of the  $^1\text{H}$ -NMR spectrum. Cyclization of **4** was accomplished using potassium carbonate as the base to afford the target scaffold **5**.



**Figure IX. Synthesis of scaffold 4.** Reagents and conditions: (a) *n*-BuLi, THF, 0 °C, 1 h, yield: 79.38%. (b) HClO<sub>4</sub>, Pd/C, H<sub>2</sub>(g), MeOH, 25 °C, 24 h, yield: 74.47%. (c) chloroacetonitrile, BCl<sub>3</sub>, AlCl<sub>3</sub>, DCM, 25 °C, 24 h yield: 69.75%. (d) K<sub>2</sub>CO<sub>3</sub>, DMF, 25 °C, 1 h, yield: 51.71%.

The advantages of this synthetic pathway were clear: firstly, scaffold **5** was synthesized in 4 reaction steps with an overall yield of 22%. Secondly, the only product which required column chromatography purification was the Sugasawa acylation product **4**, and thirdly, analogs of **5** with different alkyl substitutions could be synthesized using the same process with alternate nucleophiles or starting material aldehydes.

With the successful synthesis of the new scaffold **5**, we turned our attention to analog synthesis. It was envisioned that the final target analogs could be obtained rapidly using an aldol condensation reaction followed by selective 1,2-reduction of the resultant ketone using Luche reduction conditions (**Figure X**).

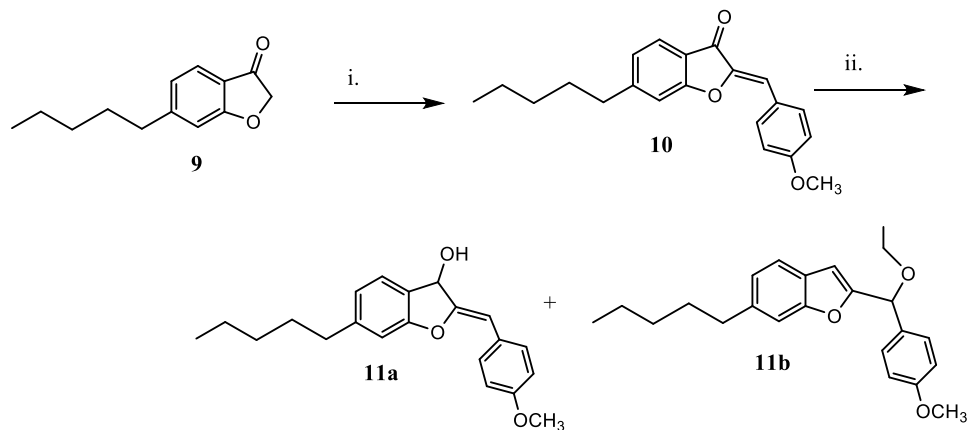


**Figure X. Synthesis of analogs from 7-pentylbenzofuran-3(2H)-one.** Reagents and conditions: (i) acetonitrile and ethylenediamine diacetic acid (EDDA), aldehyde building block, microwave (1250W power), 2 min. (ii.) NaBH<sub>4</sub>, CeCl<sub>3</sub>·7 H<sub>2</sub>O, methanol or 95% ethanol, 25°C, 20 min.

The aldol condensation reaction of **5** and substituted aldehydes was performed in the presence of ethylenediamine acetate (EDDA) under microwave conditions to afford benzofuran-3(2H)-ones (**6a-b**) in reasonable yields. Aqueous workup was not required prior to purification, as the crude reaction mixtures are typically viscous oils; simple dilution with ethyl acetate and direct loading onto a silica gel column was required for purification.

The Luche reduction (NaBH<sub>4</sub>, CeCl<sub>3</sub>·7 H<sub>2</sub>O) of benzofuran-3(2H)-ones (**6a**; R=F) resulted in the formation of the desired allyl alcohol (**7a**). When benzofuran-3(2H)-one **6b** (R=NHAc) was subjected to the Luche reduction conditions, the allylic alcohol product **7b** in addition to the rearrangement product **8** was isolated, presumably from the nucleophilic addition of ethanol to the exo-methylene of **6b** via an SN2' mechanism.

One additional set of analogs were also synthesized from the 6-pentylbenzofuran-3(2H)-one scaffold **9** previously synthesized by Dr. Eric Bow in the Rimoldi lab. Briefly, compound **9** was subjected to the aldol condensation with 4-methoxybenzaldehyde under microwave heating to afford condensation product **10**. Luche reduction of **10** using 95% ethanol as the solvent afforded a mixture of products (**11a** and **11b**) in modest yields.



**Figure XI. Synthesis of analogs from 6-pentylbenzofuran-3(2H)-one (**9**).** Reagents and conditions: (i) acetonitrile and ethylenediamine diacetic acid (EDDA), 4-methoxybenzaldehyde, microwave (1250W power), 2 min. (ii.) NaBH<sub>4</sub>, CeCl<sub>3</sub>·7 H<sub>2</sub>O, methanol or 95% ethanol, 25°C, 20 min.



## Materials and Methods

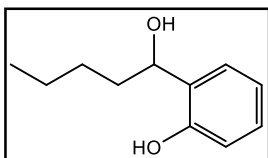
### General Methods

All reactions were monitored for starting material consumption or product formation using thin-layer chromatography (TLC) techniques and visualized under UV light (254 nm) or stained with ethanolic *p*-anisaldehyde, potassium permanganate or phosphomolybdic acid. Reactants, reagents, and solvents were purchased from Reactions performed under “standard anhydrous conditions” refers to reactions that employed flame-dried glassware evacuated and purged with an inert blanket of argon, and using commercially available anhydrous solvents. Low resolution molecular weight analysis was performed using a Waters Micromass ZQ single quadrupole mass spectrometer with either positive (ESI+) or negative (ESI-) electrospray ionization. A Bruker 400 MHz Avance Nuclear Magnetic Resonance (NMR) spectrometer was used to acquire proton ( $^1\text{H}$ ) and carbon ( $^{13}\text{C}$ ) NMR spectral data, and the data processed using MNova (MestReNova) software. Ligands were assessed for putative receptor affinity using a cannabinoid receptor binding assay conducted by COBRE Core C at the University of Mississippi. The analogs synthesized were tested in three solutions of differing concentrations (0.5  $\mu\text{M}$ , 2.5  $\mu\text{M}$ , 10  $\mu\text{M}$ ) and run in a competitive radioligand binding assay against both the CB<sub>1</sub> and CB<sub>2</sub> receptors. The percent displacement of the compounds was calculated by considering both the binding of the analog (specific

binding) and the nonspecific binding using the following formula:  $100 - (\text{binding of compound} - \text{nonspecific binding}) \times (100/\text{specific binding})$ .<sup>4</sup>

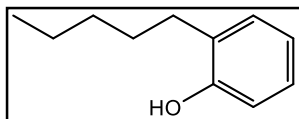
## Experimental Methods

### (2) 2-(1-hydroxypentyl)phenol



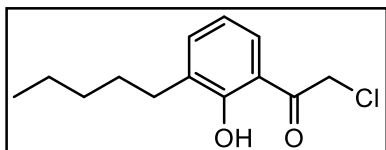
Under standard anhydrous conditions, commercially available 2-hydroxy-benzaldehyde (**1**, 872.6  $\mu\text{L}$ , 8.19 mmol) was dissolved in THF (15 mL) and cooled to 0°C. *n*-Butyllithium (1 mL, 16.38 mmol) was slowly added dropwise to the solution. The reaction stirred for one hour, and the solution was diluted with diethyl ether, and quenched carefully with methanol (2 mL). Water was added and the reaction mixture was placed in a separatory funnel and extracted 3x with 20 mL of ethyl acetate. The organic layers were combined and washed with water and brine. The organic layer was dried over magnesium sulfate and concentrated *in vacuo* to give 1.17g (79.38%) of **2**. **MS (ESI+)**  $m/z = 203.28$   $[\text{M}+\text{Na}]^+$ . **<sup>1</sup>H NMR** (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.12 (s, 1H), 7.16 (t,  $J = 7.7$  Hz, 1H), 6.94 (d,  $J = 7.5$  Hz, 1H), 6.84 (q,  $J = 7.1$  Hz, 2H), 4.80 (t,  $J = 6.6$  Hz, 1H), 3.16 (s, 1H), 1.97 – 1.72 (m, 2H), 1.31 (ddd,  $J = 34.3, 11.4, 4.4$  Hz, 4H), 0.91 (t,  $J = 6.1$  Hz, 3H). **<sup>13</sup>C NMR** (101 MHz,  $\text{CDCl}_3$ )  $\delta$  155.37, 128.72, 127.70, 127.20, 119.68, 117.01, 76.00, 36.90, 27.88, 22.48, 13.98.

### (3) 2-pentylphenol



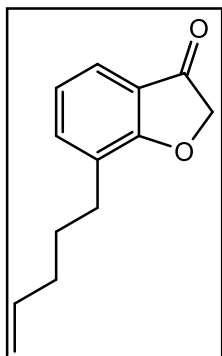
5% Pd/C (100 mg) and 60 mL of methanol were added to separate Erlenmeyer flasks. 30 mL of methanol was poured into the flask containing the Pd/C and then transferred to a round-bottomed flask containing compound **2** (1.17g, 6.50 mmol) and repeated with the remaining 30 mL of methanol. Three drops of perchloric acid was added to the mixture and stirring was initiated. The reaction flask was then purged of air and subsequently saturated with hydrogen gas. After 24 hours, the reaction was halted by filtration of the Pd/C, rinsing the catalyst repeatedly with methanol, and concentrating *in vacuo* until approximately 90% of the methanol was evaporated. The product was extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, and concentrated *in vacuo* to give 1.07 g (74.49%) of **3**. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.19 (d, *J* = 7.4 Hz, 1H), 7.13 (t, *J* = 7.7 Hz, 1H), 6.93 (t, *J* = 7.4 Hz, 1H), 6.83 (d, *J* = 7.9 Hz, 1H), 5.78 (s, 1H), 2.75 – 2.61 (m, 2H), 1.70 (p, *J* = 7.4 Hz, 2H), 1.47 – 1.37 (m, 4H), 0.98 (t, *J* = 6.4 Hz, 3H). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 172.37, 153.68, 130.18, 128.98, 126.92, 120.60, 115.27, 77.46, 77.14, 76.82, 60.97, 31.81, 29.99, 29.56, 22.65, 21.09, 14.13, 14.08.

**(4) 2-chloro-1-(2-hydroxy-3-pentylphenyl)ethan-1-one**



Compound **3** (795.30 mg, 4.87 mmol) was dissolved in 5 mL of anhydrous dichloromethane (DCM). Boron trichloride (680.08 mg, 5.80 mmol) was added to a separate round-bottomed flask, cooled to 0 °C, and the prepared solution of compound **3**, chloroacetonitrile (438.67 mg, 5.81 mmol), and aluminum trichloride (322.28 mg, 2.42mmol) were added successively. The reaction was allowed to warm to room temperature and stirred for 24 hours. The reaction was neutralized with 30 mL of 2 N hydrochloric acid and allowed to stir for one hour. The organic phase was extracted with DCM, dried over magnesium sulfate and concentrated *in vacuo*. Silica gel column chromatography purification (20% ethyl acetate/hexanes) yielded 813.0 mg (69.32%) of **4**. **MS (ESI+)**  $m/z = 263.08$   $[M+Na]^+$ . **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  12.01 (s, 1H), 7.55 (dd,  $J = 8.1, 1.5$  Hz, 1H), 7.46 – 7.35 (m, 1H), 6.92 – 6.82 (m, 1H), 4.74 (s, 2H), 2.73 – 2.62 (m, 2H), 1.69 – 1.59 (m, 2H), 1.35 (dt,  $J = 7.3, 3.8$  Hz, 4H), 0.96 – 0.83 (m, 3H). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  137.30, 136.39, 132.76, 127.08, 118.72, 63.59, 45.43, 31.67, 29.47, 28.97, 22.53, 14.03.

**(5) 7-pentylbenzofuran-3(2H)-one**



Compound **4** (813.0 mg, 3.38 mmol) and potassium carbonate (933.0 mg, 6.75 mmol) were added to 10 mL of anhydrous DMF. After 3 hours, the reaction was diluted with water and extracted with ethyl acetate and washed with water and brine. The organic phase was dried over magnesium sulfate and concentrated *in vacuo*. Silica gel column chromatography purification (20% ethyl acetate/hexanes) yielded 356.70 mg (51.71%) of **5**. **MS (ESI+)**  $m/z = 227.11$   $[M+Na]^+$ . **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.50 (d,  $J = 7.7$  Hz, 1H), 7.42 (d,  $J = 7.2$  Hz, 1H), 7.01 (t,  $J = 7.5$  Hz, 1H), 4.62 (s, 2H), 2.72 – 2.62 (m, 2H), 1.66 (dt,  $J = 13.7, 8.3$  Hz, 3H), 1.36 (dt,  $J = 7.0, 4.3$  Hz, 5H), 0.91 (t,  $J = 5.6$  Hz, 4H). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  200.56, 172.61, 137.38, 128.72, 121.92, 121.30, 120.67, 74.60, 31.52, 29.06, 28.68, 22.46, 14.01.

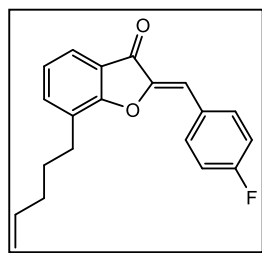
**General Procedure A: Aldol Condensations**

Scaffold **5** or **9** (25.0 mg) was added to a glass vial with 1 equivalent of desired benzaldehyde and 1 equivalent of ethylenediamine diacetic acid (EDDA), dissolved in 2 mL acetonitrile (ACN). The vial was then microwaved at 50% power (1250W) for 2 minutes followed by an additional 30-60 seconds, if necessary, to ensure consumption of starting material by TLC. The reaction was then purified using silica gel column chromatography.

## General Procedure B: Luche Reductions

The purified aldol condensation product was dissolved in either methanol or 95% ethanol and was subsequently combined with 1.1 equivalents of cerium (III) chloride heptahydrate and chilled to 0°C. 1.1 equivalent of sodium borohydride was added slowly to the solution in equal portions. The reaction was quenched slowly with water and extracted using ethyl acetate. The organic phase was washed with brine and dried over magnesium sulfate, and concentrated *in vacuo*. The crude product was purified using silica gel column chromatography (ethyl acetate/hexanes mobile phase).

### (6a) (Z)-2-(4-fluorobenzylidene)-7-pentylbenzofuran-3(2H)-one



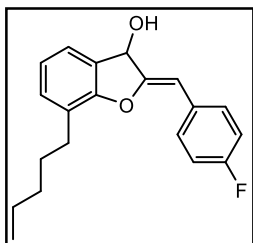
Synthesized using General Procedure A and 1 equivalent of 4-fluorobenzaldehyde. Yield: 73.71% **MS (ESI+)**  $m/z = 333.32$

$[M+Na]^+$ .  **$^1H$  NMR** (400 MHz,  $CD_3CN$ )  $\delta$  8.06 – 7.91 (m, 2H), 7.57 (d,  $J = 7.4$  Hz, 2H), 7.22 (dt,  $J = 15.2, 8.2$  Hz, 3H), 6.82 (s,

1H), 2.90 – 2.80 (m, 2H), 1.81 – 1.69 (m, 2H), 1.45 – 1.34 (m, 5H), 0.93 (t,  $J = 7.0$  Hz, 4H).

**$^{13}C$  NMR** (101 MHz,  $CD_3CN$ )  $\delta$  184.50, 164.45, 146.66, 137.13, 133.54, 133.46, 128.03, 123.70, 121.45, 116.09, 115.87, 110.36, 31.20, 29.03, 28.34, 22.14, 13.32.

**(7a) (Z)-2-(4-fluorobenzylidene)-7-pentyl-2,3-dihydrobenzofuran-3-ol**



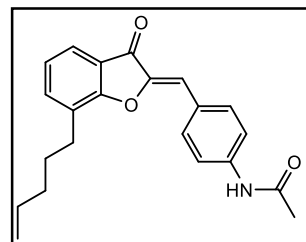
Synthesized using General Procedure B and 95% ethanol as solvent.

Yield: 46.86% **MS (ESI+)**  $m/z = 335.29$   $[M+Na]^+$ .  **$^1H$  NMR** (400

MHz,  $CDCl_3$ )  $\delta$  7.71 (dd,  $J = 8.5, 5.7$  Hz, 2H), 7.34 (d,  $J = 7.3$  Hz, 1H), 7.18 (d,  $J = 7.5$  Hz, 1H), 7.06 (dt,  $J = 18.8, 8.1$  Hz, 4H), 5.98

(s, 1H), 5.76 (s, 1H), 2.78 – 2.71 (m, 2H), 1.73 (q,  $J = 7.1$  Hz, 3H), 1.45 – 1.39 (m, 4H), 0.95 (t,  $J = 6.8$  Hz, 4H).  **$^{13}C$  NMR** (101 MHz,  $CDCl_3$ )  $\delta$  162.68, 156.67, 155.81, 130.91, 130.17, 130.09, 126.35, 125.71, 122.92, 122.90, 115.44, 115.23, 104.60, 72.83, 31.66, 29.76, 29.28, 22.46, 14.06.

**(6b) (Z)-N-(4-((3-oxo-7-pentylbenzofuran-2(3H)ylidene)methyl)phenyl)acetamide**

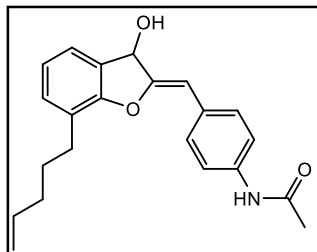


Synthesized using General Procedure A and 1 equivalent of 4-acetamidobenzaldehyde. Yield: 69.44% **MS (ESI+)**  $m/z =$

372.33  $[M+Na]^+$ .  **$^1H$  NMR** (400 MHz,  $CDCl_3$ )  $\delta$  7.71 (d,  $J =$  8.3 Hz, 2H), 7.57 (d,  $J = 7.8$  Hz, 2H), 7.43 (d,  $J = 7.4$  Hz, 1H),

7.30 (d,  $J = 7.0$  Hz, 1H), 6.97 (t,  $J = 7.3$  Hz, 1H), 6.67 (s, 1H), 5.18 (s, 2H), 2.67 (t,  $J =$  7.4 Hz, 2H), 2.05 (s, 3H), 1.61 (s, 2H), 1.25 (s, 4H), 0.76 (t,  $J = 5.9$  Hz, 3H).  **$^{13}C$  NMR** (101 MHz,  $CDCl_3$ )  $\delta$  207.07, 164.23, 146.22, 140.18, 136.58, 132.25, 127.66, 123.22, 121.58, 121.11, 119.49, 112.46, 53.41, 31.34, 28.95, 28.72, 24.23, 22.24, 13.80.

**(7b) (Z)-N-(4-((3-hydroxy-7-pentylbenzofuran-2(3H)-ylidene)methyl)phenyl)acetamide**

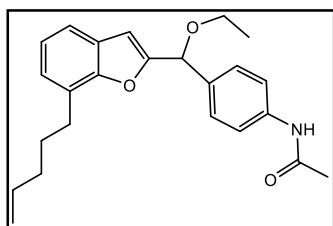


Synthesized using General Procedure B and methanol as solvent. Yield: 14.49% **MS (ESI+)**  $m/z = 374.37$   $[M+Na]^+$ . **<sup>1</sup>H**

**NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.54 (d,  $J = 3.3$  Hz, 4H), 7.33 (d,  $J = 7.4$  Hz, 1H), 7.08 (dd,  $J = 17.2, 7.4$  Hz, 2H), 6.52 (s, 1H),

5.66 (s, 1H), 2.86 (t,  $J = 6.8$  Hz, 2H), 2.18 (s, 4H), 1.78 – 1.70 (m, 2H), 1.40 – 1.32 (m, 5H), 0.89 (t,  $J = 6.7$  Hz, 4H).

**(8) N-(4-(ethoxy(7-pentylbenzofuran-2-yl)methyl)phenyl)acetamide**



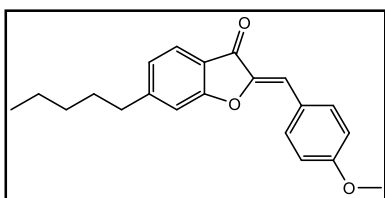
Synthesized using General Procedure B and 95% ethanol as solvent. Yield: 32.26% **MS (ESI+)**  $m/z = 402.71$   $[M+Na]^+$ .

**<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.64 (s, 1H), 7.52 (d,  $J = 8.5$  Hz, 2H), 7.44 (d,  $J = 8.4$  Hz, 2H), 7.38 – 7.31 (m, 1H), 7.12

(d,  $J = 7.4$  Hz, 1H), 7.06 (d,  $J = 7.2$  Hz, 1H), 6.53 (s, 1H), 5.49 (s, 1H), 3.72 – 3.56 (m, 2H), 3.49 (s, 1H), 2.87 (t,  $J = 7.4$  Hz, 2H), 2.16 (s, 3H), 1.72 (p,  $J = 7.3$  Hz, 3H), 1.34 (dt,  $J = 7.0, 3.8$  Hz, 5H), 1.32 – 1.25 (m, 4H), 0.90 (t,  $J = 5.6$  Hz, 4H). **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  168.54, 156.95, 153.87, 137.75, 135.20, 127.93, 127.65, 126.51, 124.07, 122.72, 119.79, 118.45, 104.85, 77.37, 77.06, 76.74, 64.97, 31.59, 29.62, 29.38, 24.51, 22.49, 15.30, 14.01.



**(10) (Z)-2-(4-methoxybenzylidene)-6-pentylbenzofuran-3(2H)-one**

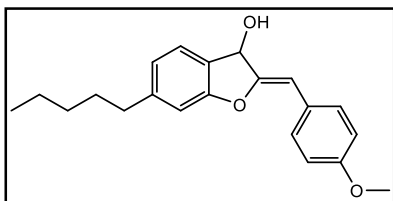


Synthesized using General Procedure A, scaffold **9**, and 1 equivalent of 4-methoxybenzaldehyde. Yield: 35.69%

**MS (ESI+)**  $m/z = 345.51$   $[M+Na]^+$ . **<sup>1</sup>H NMR** (400 MHz,

$CDCl_3$ )  $\delta$  7.87 (d,  $J = 8.9$  Hz, 2H), 7.69 (d,  $J = 7.8$  Hz, 1H), 7.13 (s, 1H), 7.02 (d,  $J = 8.9$  Hz, 1H), 6.97 (d,  $J = 8.9$  Hz, 2H), 6.84 (s, 1H), 3.86 (s, 3H), 2.79 – 2.67 (m, 2H), 1.67 (t,  $J = 11.2$  Hz, 2H), 1.35 (dt,  $J = 7.6, 3.8$  Hz, 5H), 0.94 – 0.87 (m, 3H). **<sup>13</sup>C NMR** (101 MHz,  $CDCl_3$ )  $\delta$  184.15, 166.40, 160.89, 153.68, 146.36, 133.26, 125.18, 124.18, 124.09, 119.72, 114.43, 112.79, 112.29, 55.35, 36.79, 31.37, 30.67, 22.48, 14.19, 13.97.

**(11a) (Z)-2-(4-methoxybenzylidene)-6-pentyl-2,3-dihydrobenzofuran-3-ol**

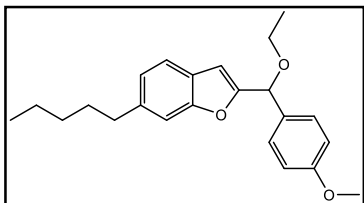


Synthesized using General Procedure B and 95% ethanol as solvent. Yield: 5.28% **MS (ESI+)**  $m/z = 347.43$

$[M+Na]^+$ . **<sup>1</sup>H NMR** (400 MHz,  $CDCl_3$ )  $\delta$  7.42 (dd,  $J =$

8.2, 4.5 Hz, 3H), 7.28 (s, 1H), 7.05 (d,  $J = 8.7$  Hz, 1H), 6.94 (d,  $J = 8.6$  Hz, 2H), 6.49 (s, 1H), 5.91 (s, 1H), 3.84 (s, 3H), 2.76 – 2.67 (m, 2H), 1.66 (dt,  $J = 15.1, 7.5$  Hz, 3H), 1.43 – 1.31 (m, 5H), 0.91 (t,  $J = 6.8$  Hz, 4H).

**(11b) 2-(ethoxy(4-methoxyphenyl)methyl)-6-pentylbenzofuran**



Synthesized using General Procedure B and 95% ethanol as solvent. Yield: 17.21% **MS (ESI+)**  $m/z = 375.42$

$[M+Na]^+$ .  **$^1H$  NMR** (400 MHz,  $CDCl_3$ )  $\delta$  7.45 – 7.36 (m, 3H), 7.03 (dd,  $J = 7.9, 1.3$  Hz, 1H), 6.92 (d,  $J = 8.8$  Hz, 2H), 6.50 (s, 1H), 5.46 (s, 1H), 3.83 (s, 3H), 3.70 – 3.54 (m, 2H), 2.74 – 2.63 (m, 2H), 1.65 (dt,  $J = 15.0, 7.5$  Hz, 2H), 1.38 – 1.32 (m, 3H), 1.30 (t,  $J = 7.0$  Hz, 5H), 0.90 (t,  $J = 6.9$  Hz, 4H).  **$^{13}C$  NMR** (101 MHz,  $CDCl_3$ )  $\delta$  159.43, 157.14, 155.58, 139.54, 131.38, 128.54, 125.72, 123.48, 120.39, 113.82, 110.97, 104.41, 77.26, 64.76, 55.28, 36.08, 31.52, 31.40, 22.55, 15.29, 14.02.

**Biological Evaluation (COBRE-NPN CORE C)**

Cannabinoid receptor assay were conducted by COBRE Core C at The University of Mississippi. Assays were conducted in accordance with the method republished below in Tarawneh et al. "Reprinted with permission from Tarawneh, A. et al., Flavonoids from *Perovskia atriplicifolia* and Their in Vitro Displacement of the Respective Radioligands for Human Opioid and Cannabinoid Receptors. *Journal of Natural Products*, 2015. 78(6): p. 1461. Copyright (2015) American Chemical Society."

### **Cell Culture and Membrane Preparation.**

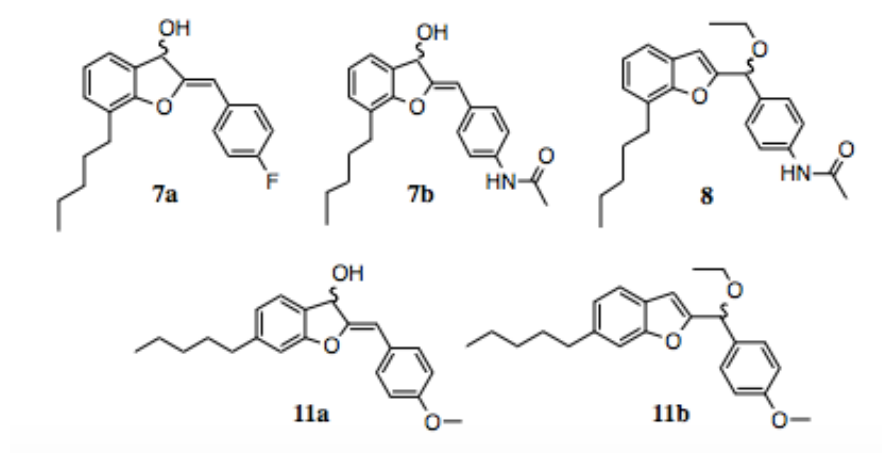
HEK293 cells (ATCC) were stably transfected with plasmids containing cloned humancannabinoid receptor subtypes 1 and 2 (obtained from Origene, Rockville, MD, USA). These cells were maintained in a humidified incubator at 37°C and 5% CO<sub>2</sub> in a Dulbecco's modified Eagle's medium (DMEM) nutrient mixture F-12 HAM supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1000 IU/mL penicillin, and 1000 µg/mL of streptomycin, and 0.5 mg/mL G418 antibiotic solution. Membranes for the radioligand binding assays were prepared by scraping the cells in cold Tris-HCl, pH 7.4, and then centrifuged at 5200 g for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in the same buffer, homogenized using a sonic dismembrator model 100 (Fisher Scientific, Pittsburgh, PA, USA) for 30 seconds, and then centrifuged at 1000g for 10 min at 4°C. The supernatant was saved, and the pellet underwent the suspension and sonication process two additional times under the same conditions. The supernatants were combined and centrifuged at 23300g for 40 minutes at 4°C. The pellet was resuspended and aliquoted into 2 mL vials and stored at -80 °C. The total protein concentration was determined using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions. The optimal membrane and radioligand concentrations for each receptor batch were established through membrane evaluation and saturation binding experiments.

### **Radioligand Displacement Assay for Cannabinoid Receptor Subtypes.**

Compounds evaluated in this assay were run in competition binding with both cannabinoid receptor subtypes, CB1 and CB2. CB receptor binding screening was performed under the following conditions: 10  $\mu$ M of each compound from independent triplicate dilutions was incubated with 1.6975 nM (CB1) or 1.959 nM (CB2). [3H]-CP 55,940, and 5  $\mu$ g of CB1 or 1  $\mu$ g of CB2 membrane were incubated for 90 min at 37°C with gentle agitation in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, 20 mM EDTA, 154 mM NaCl, and 0.2% radioimmunoassay grade BSA, pH 7.4. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/C filter (PerkinElmer Life Sciences Inc., Boston, MA, USA), presoaked with 0.3% polyethylenimine, followed by 10 washes with 50 mM Tris-HCl, pH 7.4, buffer containing 0.2% BSA. Filters were dried, 25  $\mu$ L of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter (PerkinElmer Life Sciences Inc., Boston, MA, USA). Total binding was defined as binding in the presence of vehicle (1.0% DMSO). Nonspecific binding was the binding observed in the presence of 10.0  $\mu$ M CP-55,940. Specific binding was defined as the difference between total and nonspecific binding. Percent displacement was calculated using the following formula:  $100 - (\text{binding of compound} - \text{nonspecific binding}) \times (100 / \text{specific binding})$ .

## Results

Three target analogs were successfully synthesized from the 7-pentylbenzofuran-3(2H)-one (**5**), namely the (Z)-2-benzylidene-7-pentyl-2,3-dihydrobenzofuran-3-ols (**7a** and **7b**) and the rearrangement product **8**. Two additional analogs containing a 4-methoxyphenyl group (**11a** and **11b**), and were synthesized from 6-pentylbenzofuran-3(2H)-one (**9**) (**Figure XII**). The facile synthesis of these scaffolds allowed for the implementation of an aldol condensation reaction, performed under microwave heating, and the Luche reduction reaction, which resulted in some instances, rearrangement products containing ethanol adducts. The Luche reduction of **10** was unique insofar as it resulted in sufficient yields of both the allylic alcohol product and rearrangement product for testing. The structures of the final products and intermediates were confirmed using NMR spectrometry.

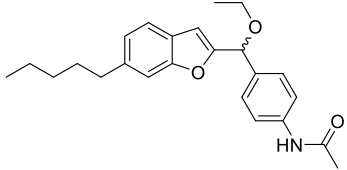
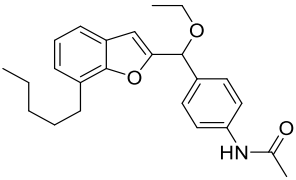
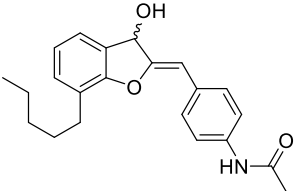


**Figure XII. Series of synthesized ligands subjected to CB binding assay.**

	CB1 % Displacement			CB2 % Displacement		
	10 $\mu$ M	2.5 $\mu$ M	0.5 $\mu$ M	10 $\mu$ M	2.5 $\mu$ M	0.5 $\mu$ M
<b>7a</b>	24.3	17.6	14.8	43.3	30.5	14.0
<b>8</b>	59.0	44.9	37.7	83.4	62.2	26.3
<b>7b</b>	0	13.0	15.2	20.1	15.2	15.8
<b>11a</b>	57.1	38.0	37.7	68.9	48.8	32.0
<b>11b</b>	71.9	57.0	36.8	79.6	78.9	<b>56.2</b>

**Table I. CB binding data**

CB binding assay data results demonstrated that compound **11b** had the highest affinity of the five compounds tested (at 500 nM concentration). Both 4-methoxyphenyl derivatives derived from the 6-pentyl substituted scaffold, **11a** and **11b** displayed better binding affinity with a slight selectivity for the CB<sub>2</sub> receptor, more so than with ethoxy rearrangement. Compared to previously tested compound (**3.4**), compounds **7b** and **8** did not bind as effectively, suggesting that 7-substitued alkyl chains have a higher affinity/better fit for both CB receptors. However, **7b** and **8** seem to favor binding to the CB<sub>2</sub> receptor in higher concentrations. In addition, the allylic alcohol product seems to be the least favorable structure for binding affinity (**Table II**).

	CB1 % Displacement			CB2 % Displacement		
	10 uM	2.5 uM	0.5 uM	10 uM	2.5 uM	0.5 uM
 <b>3.4<sup>4</sup></b>	94.5	*	31.9	96.7	*	57.7
 <b>8</b>	59.0	44.9	37.7	83.4	62.2	26.3
 <b>7b</b>	-	13.0	15.2	20.1	15.2	15.8

**Table II. Comparison of CB receptor assay binding for compounds 7b, 8 and 3.4 (synthesized by Eric Bow).** \* = not tested in corresponding concentration. - = 0% displacement.

Based on the CB receptor binding data, the analog containing the 4-methoxyphenyl group in addition to the ethoxy rearrangement (**11b**) has the greatest potential for CB<sub>2</sub> selectivity. The location of the pentyl chain has an influence on CB binding and selectivity, with 6-alkyl substitution preferred over 7-alkyl substitution. Additional analogs will be required to build a more complete structure activity relationship analysis. Although the compound data did not result in the discovery of a CB<sub>2</sub> selective compound, they were very promising and offered a direction for future work towards the discovery of CB<sub>2</sub> selective ligands.

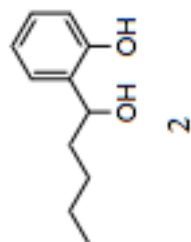
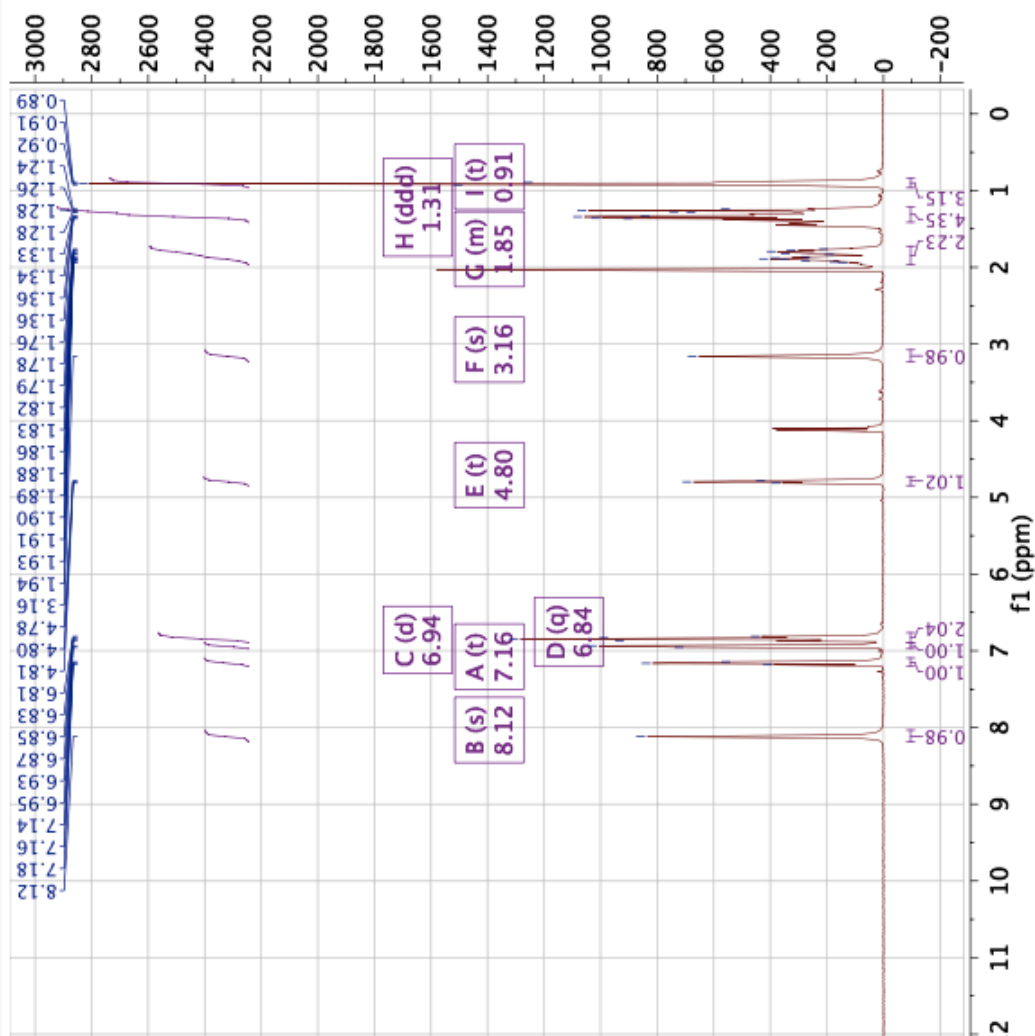


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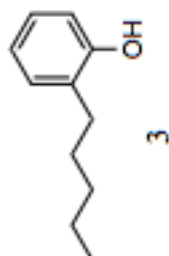
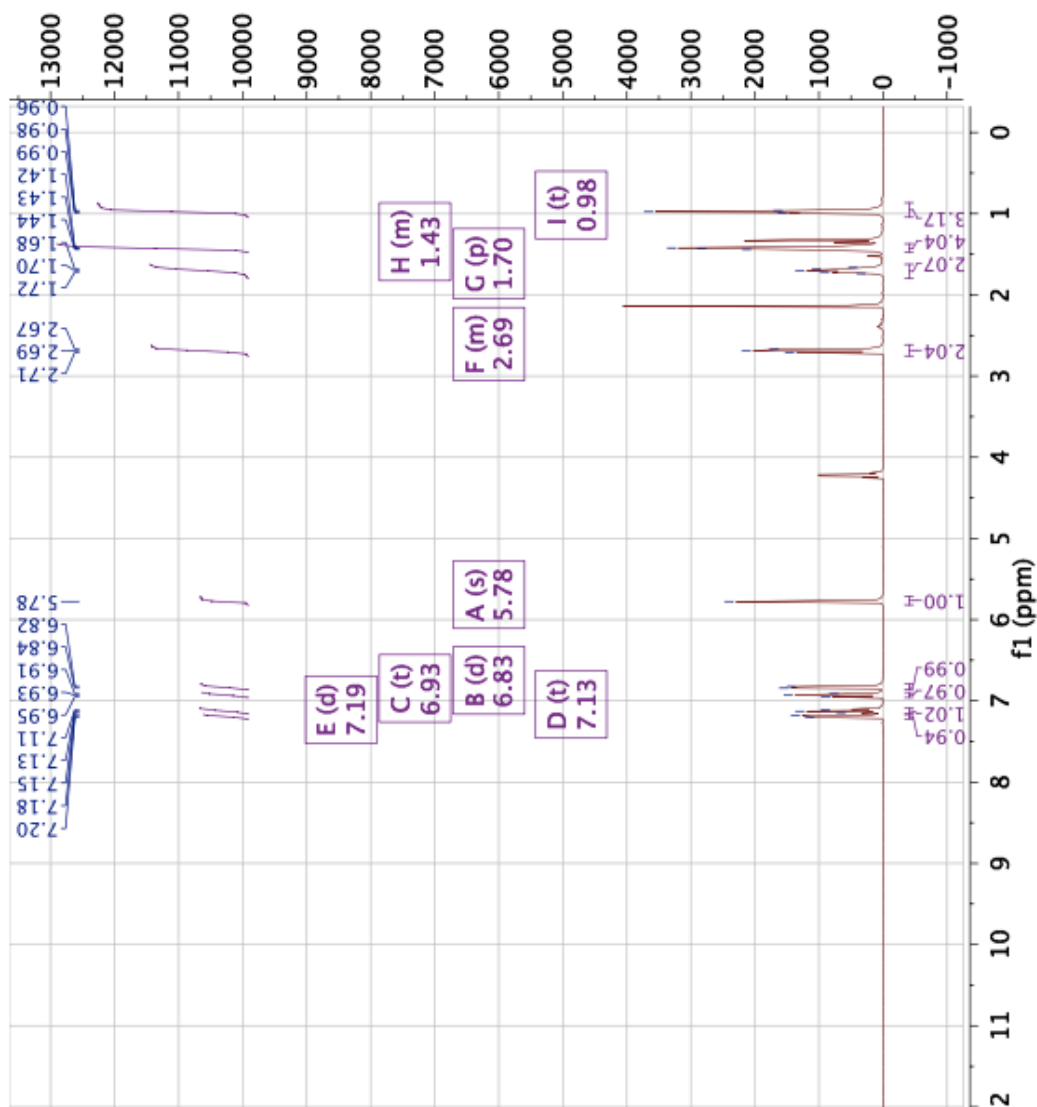
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## **APPENDIX**



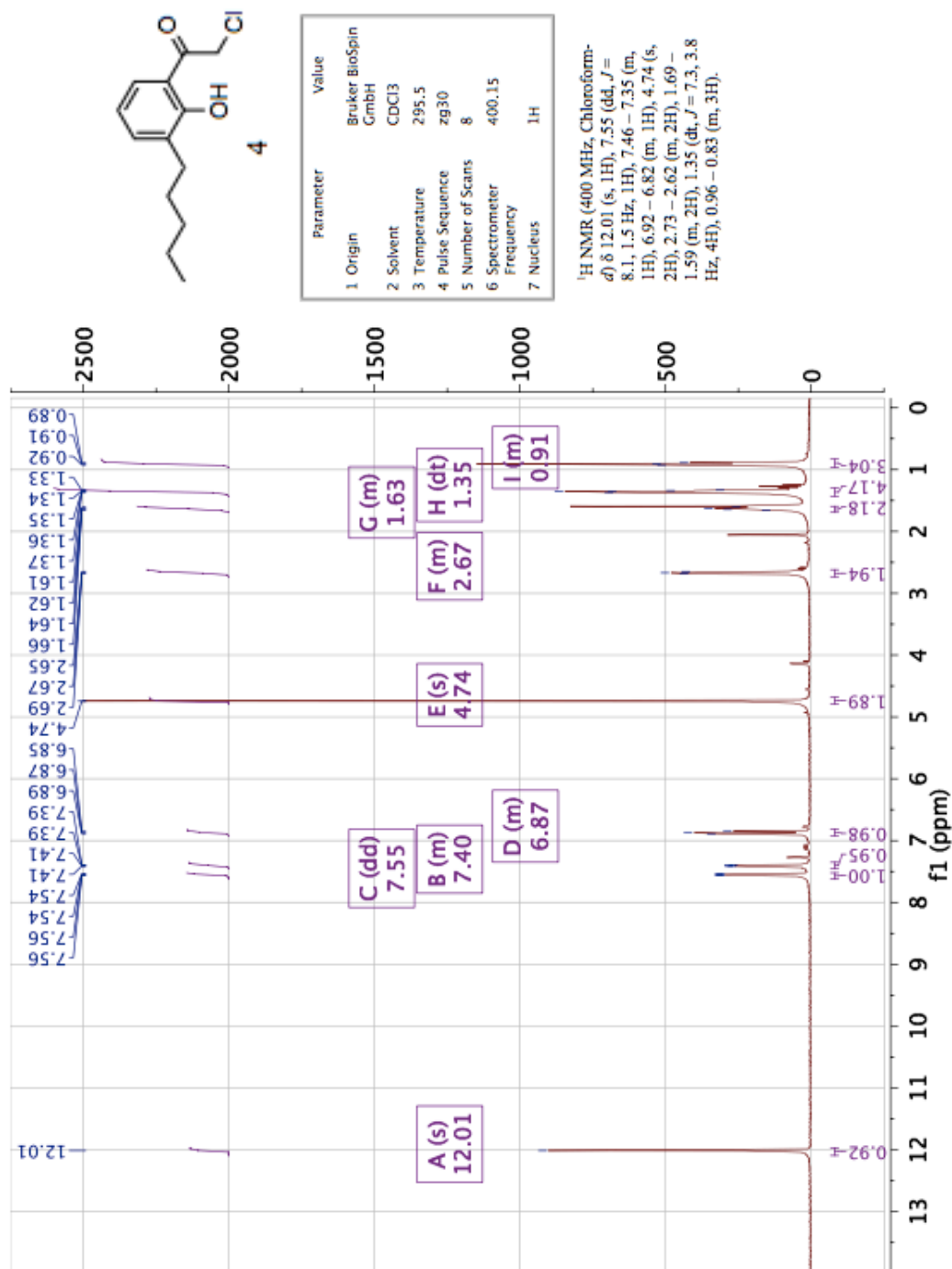
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1 Origin	Bruker BioSpin GmbH
2 Solvent	CDCl3
3 Temperature	298.2
4 Pulse Sequence	zg30
5 Number of Scans	8
6 Spectrometer Frequency	400.15
7 Nucleus	<sup>1</sup> H

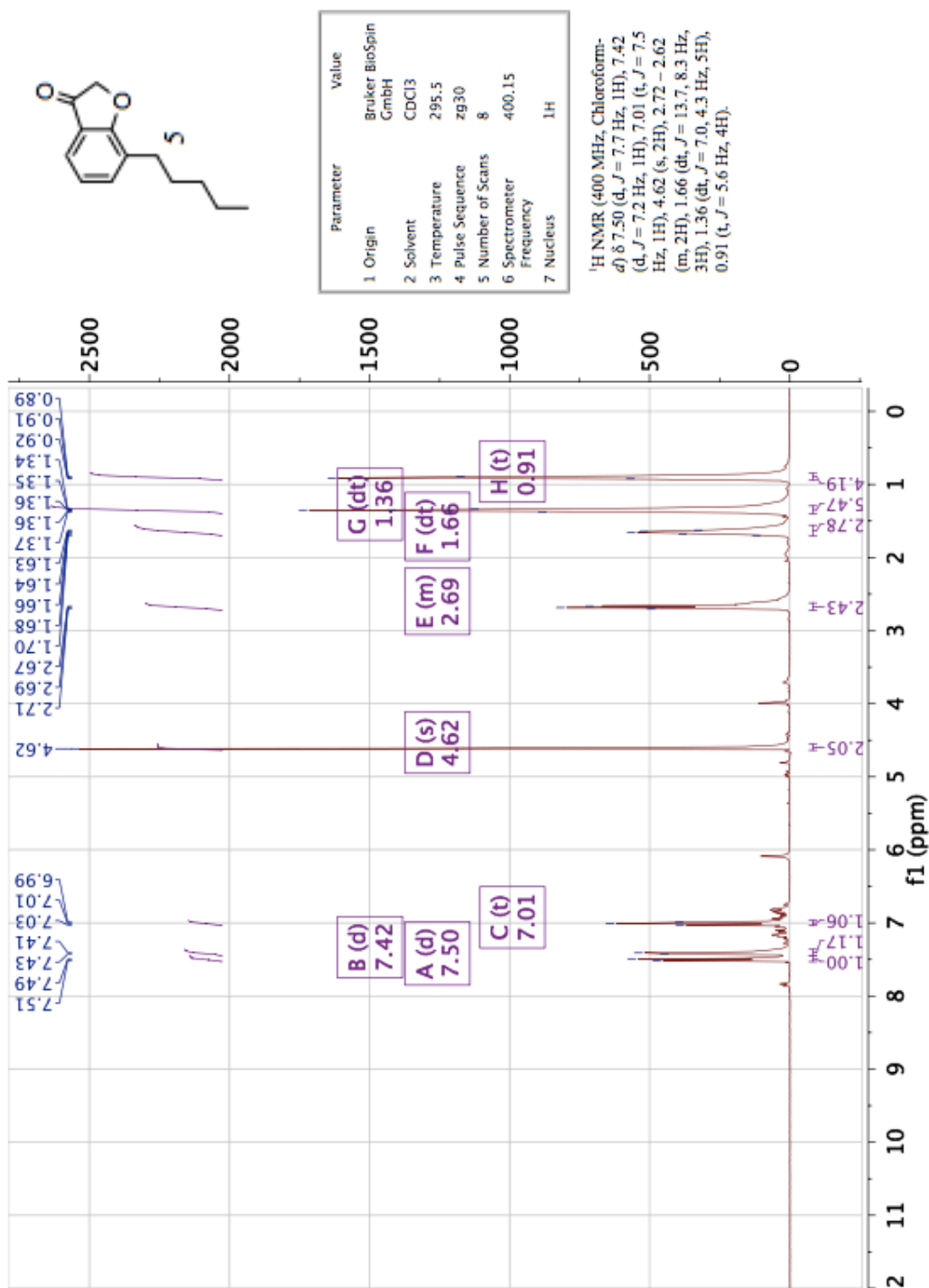
<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  8.12 (s, 1H), 7.16 (t,  $J$  = 7.7 Hz, 1H), 6.94 (d,  $J$  = 7.5 Hz, 1H), 6.84 (q,  $J$  = 7.1 Hz, 2H), 4.80 (t,  $J$  = 6.6 Hz, 1H), 3.16 (s, 1H), 1.97 – 1.72 (m, 2H), 1.31 (dd,  $J$  = 34.3, 11.4, 4.4 Hz, 4H), 0.91 (t,  $J$  = 6.1 Hz, 3H).

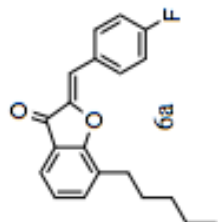
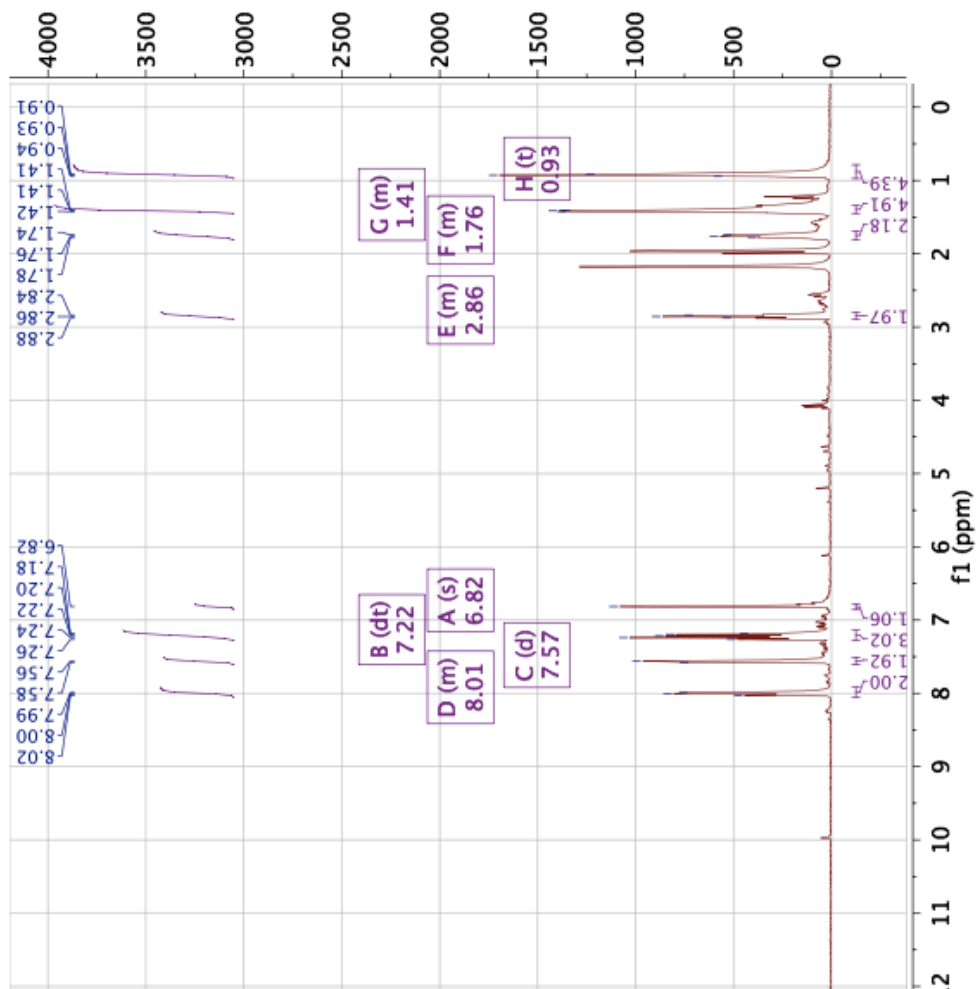


Parameter	Value
1 Origin	Bruker BioSpin GmbH
2 Solvent	CDCl <sub>3</sub>
3 Temperature	298.2
4 Pulse Sequence	zg30
5 Number of Scans	8
6 Spectrometer Frequency	400.15
7 Nucleus	<sup>1</sup> H

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  7.19 (d,  $J$  = 7.4 Hz, 1H), 7.13 (t,  $J$  = 7.7 Hz, 1H), 6.93 (t,  $J$  = 7.4 Hz, 1H), 6.83 (d,  $J$  = 7.9 Hz, 1H), 5.78 (s, 1H), 2.75 – 2.61 (m, 2H), 1.70 (p,  $J$  = 7.4 Hz, 2H), 1.47 – 1.37 (m, 4H), 0.98 (t,  $J$  = 6.4 Hz, 3H).





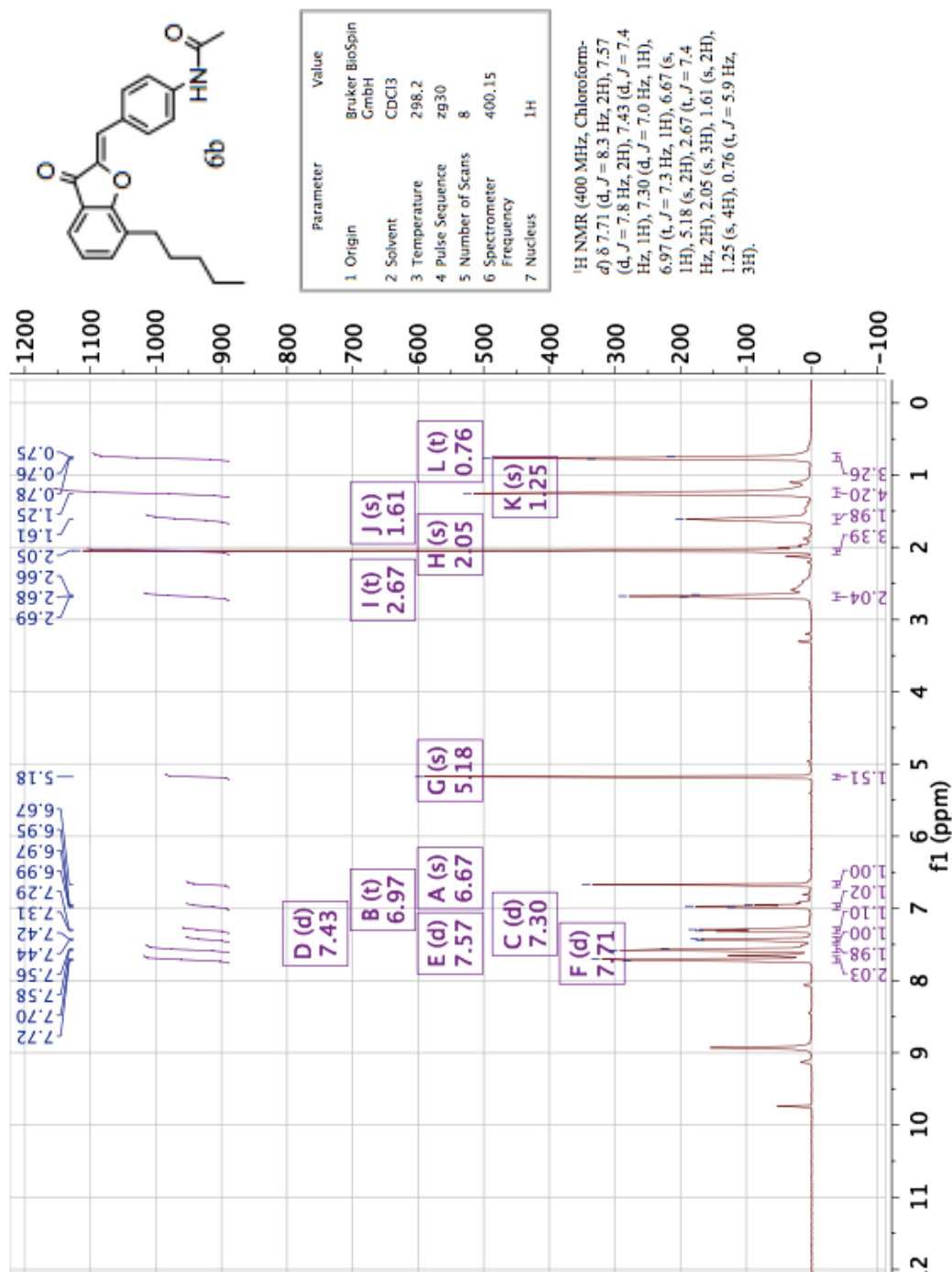


Parameter	Value
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2 Solvent	CD3CN
3 Temperature	298.1
4 Pulse Sequence	zg30
5 Number of Scans	18
6 Spectrometer Frequency	400.15
7 Nucleus	<sup>1</sup> H

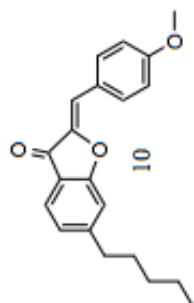
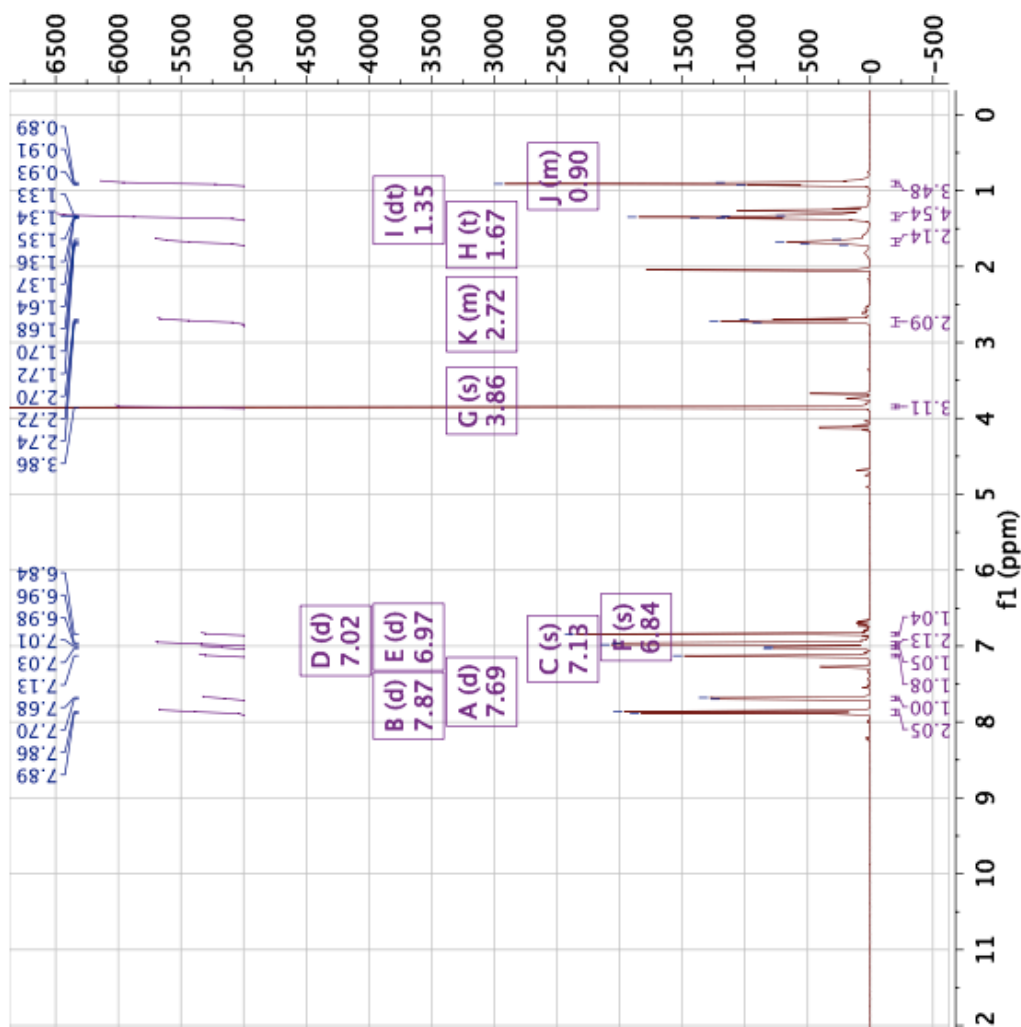
<sup>1</sup>H NMR (400 MHz, Acetonitrile-*d*<sub>3</sub>) 8.06 – 7.91 (m, 2H), 7.57 (d, *J* = 7.4 Hz, 2H), 7.22 (dt, *J* = 15.2, 8.2 Hz, 3H), 6.82 (s, 1H), 2.90 – 2.80 (m, 2H), 1.81 – 1.69 (m, 2H), 1.45 – 1.34 (m, 5H), 0.93 (t, *J* = 7.0 Hz, 4H).











Parameter	Value
1 Origin	Bruker BioSpin
2 Solvent	CmbH
3 Temperature	298.2
4 Pulse Sequence	zg30
5 Number of Scans	8
6 Spectrometer Frequency	400.15
7 Nucleus	<sup>1</sup> H

<sup>1</sup>H NMR (400 MHz, Chloroform-*d*)  $\delta$  7.87 (d, *J* = 8.9 Hz, 2H), 7.69 (d, *J* = 7.8 Hz, 1H), 7.13 (s, 1H), 7.02 (d, *J* = 8.9 Hz, 1H), 6.97 (d, *J* = 8.9 Hz, 2H), 6.84 (s, 1H), 3.86 (s, 3H), 2.79 – 2.67 (m, 2H), 1.67 (t, *J* = 11.2 Hz, 2H), 1.35 (dt, *J* = 7.6, 3.8 Hz, 5H), 0.94 – 0.87 (m, 3H).



